In Claim 39, line 1, delete "37", and insert therefor --54--.

In Claim 40, line 1, delete "36", and insert therefor --59--.

Please add the following new claims.

--~~58~~. The composition of Claim ~~38~~, wherein said polymerase is a thermostable DNA polymerase.

~~54~~. The composition of Claim ~~53~~ in a buffer with pH in the range of 3.5 to 9.5.

~~55~~. The composition of Claim ~~53~~ in a buffer with pH in the range of 4.0 to 8.5.

~~56~~. The composition of Claim ~~55~~, wherein said detergent is present in a concentration of about 0.1% to 1.0%.

~~57~~. The composition of Claim ~~56~~, wherein said DNA polymerase is isolated from a ~~Thermus~~ species of the genus Thermus.

~~58~~. The composition of Claim ~~57~~, wherein said species is selected from the group consisting of flavus, ruber, thermophilus, aquaticus, lacteus, and rubens.

~~59~~. The composition of Claim ~~57~~, wherein said species is aquaticus.

~~60~~. The composition of Claim ~~57~~, wherein said species is flavus.

~~61~~. The composition of Claim ~~57~~, wherein said species is thermophilus.

62. A reaction mixture that comprises nucleoside -5'-triphosphates, oligonucleotide primers, a buffer in which primer extension by a polymerase can occur, and an aliquot of the stable enzyme composition of Claim 1.--

Remarks

The Invention

The present invention provides a novel means for stabilizing thermostable nucleic acid polymerase compositions. The invention is especially significant in the important role played by the invention in the commercialization of PCR technology. Assignee Cetus Corporation is the recognized leader in the supply of PCR reagents and instruments. Taq polymerase is arguably the most important reagent currently used in PCR, and the present invention has contributed significantly to the success Cetus enjoys in the marketplace.

Purified Taq polymerase is the subject matter of U.S. Patent No. 4,889,818; the present application is a division of the application that matured into the '818 patent. As noted in the accompanying information disclosure statement, when Cetus' competitors began marketing Taq polymerase, those competitors quickly copied Cetus' use of stabilizers in their own products. A

recent entry into the thermostable DNA polymerase market, du Pont's polymerase isolated from Thermus flavus also refers to the presence of "stabilizers," which Applicants believe refers to the presence of non-ionic detergents in the product.

Applicants believe the commercial success enjoyed by the present invention demonstrates the non-obvious nature of the present invention. Applicants have amended the specification and claims to place the application in condition for allowance so that the invention can enjoy the protection provided by the patent laws. Applicants respectfully request favorable consideration of the application.

The Amendments to the Specification

Applicants have amended the title (and the "field of the invention") to refer to the invention claimed in this divisional application. Support for the amendment is found in the words of the claims.

Examiner should note that Applicants have also added a "Cross-Reference" in the amendment to the title. This cross-reference describes applications formerly listed at lines 3-6 of page 1.

Those lines, and lines 7-11 of that page, have been deleted. Lines 7-11 referred to "related" applications. Some of these applications (the '331 and '368 applications) are discussed elsewhere in the text. Others (the '344, '061, and '513 applications) and the '195 patent are discussed in the accompanying disclosure statement and have been deleted from the former first paragraph of the specification.

The reference to "ATCC No. 40,366" at pages 12 and 83 has been changed to "ATCC No. 40,336," as that is the correct designation of the CH35:Taq#4-2 recombinant host strain. In similar fashion, Applicants have inserted the ATCC No. for the host strain of plasmid pAW740CHB -- ATCC No. 67,605 -- at pages 64 and 83. Copies of the deposit contracts for the two strains are enclosed for the record.

Finally, Applicants have requested amendments to update the status of various patent applications referred to in the specification.

The amendments to the specification have not introduced now matter but do facilitate an understanding of the claimed invention. Applicants respectfully request entry of the amendments to the specification.

The Amendments to the Claims

Applicants have amended the claims to limit the claims to the subject matter of Invention 2, as identified by Examiner in the Office Action mailed February 1, 1990. In so doing, Applicants have not cancelled Claim 1, identified by Examiner as the subject matter of Invention 1, but instead have amended the claim to refer to "stabilized enzyme compositions."

Claims 35 and 36 have been amended to specify the molecular weight of the non-ionic polymeric detergents in the composition of Claim 1. Support for the amendment is found at page 24 of the application.

The dependency of Claim 37 has been changed from Claim 36 to Claim 35. However, as these claims have likewise been amended, amended Claim 37 is more narrow than original Claim 37. Support for the amendment is found in original Claim 37 and at page 24 of the application.

Claim 38 has been amended to recite a Markush group of non-ionic polymeric detergents. Each member of this group is found at pages 24 and 25 of the specification. Amended Claim 38 also lists each detergent found in original Claim 38.

Original Claims 39-41 have been amended to depend from additional Claims 53-61, presented in the present response. Claim 39 was changed from dependent on Claim 37 to dependent on Claim 54. For Claim 40, the dependency was changed from Claim 36 to Claim 59. Claim 41 was not amended, but, as the claim depends from Claim 40 (amended as discussed above), there is an implicit amendment to Claim 41.

Additional Claims 53-61 focus on the nature of the nucleic acid polymerase in the stabilized enzyme composition; the pH of the composition; and the detergent concentration of the composition.

Claim 53 specifies that the polymerase is a DNA polymerase. Support for the claim is found in the exemplification of stabilized Taq DNA polymerase compositions in the application.

Claims 54 and 55 specify that the pH of the buffered composition is between 3.5 and 9.5 (Claim 54) and 4.0 and 8.5 (Claim 55). Support for the amendment is found at page 24 of the application.

Claim 56 specifies a detergent concentration in the range of 0.1 to 1.0%. Support for this claim is found in original Claims 36 and 37, which specified that there could be "one or more" (i.e., two) detergents and each detergent could be present in a range from "about 0.1% to

about 0.5%." Support is also found in original Claim 4, which specifies two detergents, each present at 0.5%.

Claim 57 specifies that a Thermus DNA polymerase be present in the composition. Claim 58 lists a Markush group of Thermus species, and Claims 59-61 list specific species of the group of Claim 58. Support for these claims is found at page 8 of the application and in the exemplification of a Thermus species, aquaticus, as a preferred embodiment of the present invention.

Additional Claim 62 is drawn to a reaction mixture that comprises an aliquot of the stable enzyme composition of Claim 1. As the Examples of the present application describe, the stabilized enzyme compositions of the present invention can be used to promote the polymerization (primer extension) step of the polymerase chain reaction. Claim 62 is drawn to the resulting reaction mixtures.

Applicants believe the amended claims point out this novel invention with particularity and clear language and should be allowed.

The Restriction Requirement

For the record, Applicants elect the invention of Group II, Claims 35-41, for prosecution. As noted above, Claim 1, the sole claim in this application directed to Examiner's invention I, has been amended to recite the subject matter of original Claims 35-41. These amendments have necessitate a change in inventorship, as discussed below and in accompanying papers submitted under 37 C.F.R. §1.48.

Change in Inventorship

The stabilized enzyme compositions and reaction buffers of the invention were invented by inventors David Gelfand, Susanne Stoffel, and Randy Saiki. Inventor Frances Lawyer contributed only to the subject matter of the application pertaining to the recombinant DNA expression vectors used to produce recombinant Taq polymerase. The resulting amendment to inventorship is presented in the accompanying papers submitted under 37 C.F.R. §1.48.

Accompanying Information Disclosure Statement

Applicants have filed a disclosure statement for this application concurrently with the present response to office action. Examiner is respectfully requested to consider the information in that disclosure in deciding on the patentability of the present claims.

Conclusion

As the invention has met with commercial success and has found numerous supporters in the marketplace, Applicants deserve the patent protection sought. Favorable consideration of the application is requested.

Respectfully submitted,

By: Kevin R Kaster

Kevin R. Kaster
Attorney for Applicants
Reg. No. 32,704

May 7, 1990

CETUS CORPORATION
1400 Fifty-Third Street
Emeryville, CA 94608
(415) 420-3444